Journal of Chromatography, 235 (1982) 173–185
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,319

DETERMINATION OF NITRATE IN MEAT PRODUCTS AND CHEESES BY GAS–LIQUID CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

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(Received August 25th, 1981)

SUMMARY

A simple, sensitive and practical method for the determination of nitrate in various meat products and cheeses is described. The method is based on the nitration of 2-*sec*.-butylphenol in *ca.* 57% sulphuric acid to form 4-nitro-2-*sec*.-butylphenol. After nitration, the toluene extract is re-extracted with an alkaline solution and subsequently analysed by gas–liquid chromatography with an electron-capture detector (GLC-ECD) using a column of OV-17 on Chromosorb W HP after pentafluorobenzoylation and extraction with *n*-hexane. The nitrate concentration is calculated from the peak height. Amounts of 0.05–1.0 μg of nitrate can be determined. The detection limit for nitrate is 0.006 μg/ml. The procedure for determining nitrate in meat products and cheeses involves direct analysis by GLC-ECD without clean-up; the detection limit is 0.07 ppm and the recovery from meat products and cheeses ranged from 96.8 to 99.0% at the 5 ppm level and from 94.7 to 98.6% at the 10 ppm level. The nitrated compound of 2-*sec*.-butylphenol was identified as 4-nitro-2-*sec*.-butylphenol by thin-layer chromatography and nuclear magnetic resonance spectroscopy and the final pentafluorobenzoylated product was confirmed by combined gas chromatography–mass spectrometry.

INTRODUCTION

Nitrogen plays an important biological role in living organisms and nitrate is one of the principle nutrients for aquatic life. In recent years, increasing concern has been focused on the formation of carcinogenic nitrosoamines from various nitrogen oxides[1,2]. The development of rapid, sensitive and accurate methods for the determination of trace amounts of nitrate in environmental samples is therefore of interest. Current methods for the determination of nitrate-nitrogen include reduction to ammonia followed by titration or spectrophotometry[3,4], chemical or biochemical reduction to nitrate and its diazotization with a variety of reagents[5–8], nitration of organic reagents and determination of the nitration product[9–11], ultraviolet spectrometry[12,13], polarography[14] and ion-selective electrodes[15,16]. The highly sensitive method[5,6,8] based on copperized cadmium for reducing nitrate to nitrite and then

0021-9673/82/0000–0000/$02.75

diazotization of sulphanilamide is precise but time consuming and cadmium is highly toxic. Spectrophotometric methods[17,18] are also time consuming because of the need for a distillation stage after nitration.

Recently, the determination of nitrate by gas–liquid chromatography (GLC) has been described. Brief studies of nitration with nitrate and benzene[19] or 2,4-xylenol[20] in the presence of 80% sulphuric acid using an electron-capture detector (ECD) have been made; the procedure is complex and vigorous reaction conditions are required. Tanner *et al.*[21] applied an ECD to 4-nitro-2,3,5,6-tetrafluoroanisole after nitration of 2,3,5,6-tetrafluoroanisole. Tan[22] studied the determination of nitrate as nitrobenzene after reaction with 1,3,5-trimethoxybenzene with a multiple ion detector. These methods were not suitable for routine use because of the many interferents and the conditions required. However, we found that 4-nitro-2-*sec.*-butylphenol can be prepared quantitatively by reaction of nitrate and 2-*sec.*-butylphenol in acidic medium, followed by GLC-ECD after reaction with pentafluorobenzoyl chloride (PFB-Cl) in a weak alkaline solution. The detection limit was 0.006 μg of nitrate-nitrogen.

Nitrate in meat products and cheeses was extracted with an alkaline solution. This GLC method is a simple and highly sensitive and practical means of determining nitrate in various meat products and cheeses. The recovery of nitrate added to meat products and cheeses was satisfactory.

EXPERIMENTAL

Reagents and apparatus

Potassium nitrate was dried at 110 C for 4 h under vacuum immediately before use. A stock nitrate solution was prepared by dissolving 0.718 g of potassium nitrate in 1000 ml of distilled water to give a concentration of 1 μg/ml of nitrate-nitrogen. 2-*sec.*-Butylphenol (Tokyo Kasei Kogyo, Tokyo, Japan) was of a special high grade and used without further purification; a 5% (w/v) solution was prepared by dissolving 5.0 g in 100 ml of ethanol. Pentafluorobenzoyl chloride (Aldrich, Milwaukee, WI, U.S.A.) was of a special high grade and was used without further purification.

The internal standard solution for GLC was prepared by dissolving 1.0 μg of *p,p'*-dichlorophenyldichloroethylene (DDE) in 1 ml of *n*-hexane. Deproteinizing solution (12%, w/v) was prepared by dissolving 12 g of zinc sulphate in 100 ml of distilled water. Silver sulphate solution (5%, w/v) was prepared by dissolving 5 g of silver sulphate in 100 ml of distilled water. All water was triply distilled and deionized. The column packing materials for GLC, *viz.*, Chromosorb W HP, SE-30, OV-101, OV-17, OV-225, QF-1 and XE-60, were of high purity and were obtained from Nihon Chromato (Tokyo, Japan). All other reagents and solvents were of high purity and were obtained from Wako (Osaka, Japan).

For identification of the pentafluorobenzoylated product of 4-nitro-2-*sec.*-butylphenol, a Shimadzu LKB-9000 combined gas chromatograph–mass spectrometer was used. For GLC, a glass tube (1.5 m × 3 mm I.D.) packed with OV-17 on Chromosorb W HP (80–100 mesh) was used; the helium flow-rate was 30 ml/min and the column temperature 210°C. The conditions for mass spectrometry (MS) were as follows: separator temperature, 260°C; ion source temperature, 290°C; trap current, 60 μA; electron energy, 70 eV; and accelerating potential, 3.5 keV. Nuclear magnetic

resonance (NMR) spectra were measured at 60 Hz with a Varian EM-60 spectrometer.

Preparation of pentafluorobenzoyl ester of 4-nitro-2-sec.-butylphenol

A suitably diluted solution of nitrate was placed in a test-tube (about 20 × 2.0 cm I.D.) and made up accurately to 4 ml with distilled water, then 1 ml of 5% silver sulphate solution, 7 ml of sulphuric acid (gently) and 0.1 ml of 5% 2-*sec.*-butylphenol solution were added. After reaction at room temperature with occasional shaking for 15 min, the reaction mixture was transferred into a 50-ml separating funnel and 10 ml of toluene were added. The mixture was shaken for 5 min and the toluene layer was separated and washed twice with 10 ml of distilled water. The toluene layer was re-extracted with 10 ml of 5% sodium carbonate solution, the alkaline solution was transferred into a 50-ml separating funnel and 20 μl of PFB-Cl were added. The mixture was shaken for 1 min and allowed to stand for 5 min, then extracted with 10 ml of *n*-hexane, separated and dried with 1 g of anhydrous sodium sulphate. After addition of 1 ml of internal standard solution, a 1-μl volume of the solution was injected into the gas chromatograph.

Gas–liquid chromatography

A Shimadzu GC-4BM gas chromatograph with an ECD was used for all analyses. The column was a glass tube (1.5 m × 3 mm I.D.) packed with 5% OV-17 on Chromosorb W HP (80–100 mesh) and was conditioned at 210°C; the detector and injector temperature were 300°C. The flow-rate of the carrier gas (nitrogen) was 75 ml/min and the electrometer range was 10^2 MΩ × 0.16 V.

Calibration graph

A series of standard nitrate solutions were prepared by dilution of the stock solution. Aliquots were placed into a test-tube to give amounts of 0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 μg of NO_3-N. According to the procedure described above, 10 ml of toluene extracts were obtained in each instance, and then re-extracted with sodium carbonate solution. After pentafluorobenzoylation by addition of PFB-Cl and subsequent reaction and extraction, a 1-μl aliquots of the mixture (11 ml) was injected into the GLC column with the internal standard solution as in the described procedure. As shown in Fig. 1, the retention time of the PFB derivative relative to that of DDE was 0.64. The peak-height ratio of the PFB derivative of DDE was plotted against the amount of NO_3-N analysed; a typical calibration graph is shown in Fig. 2.

Preparation of meat products and cheese extracts and their determinations

To 10 g of finely ground sample in a 100-ml flask fitted with a ground-glass stopper were added 70 ml of hot water (70–80°C) and 2.5 ml of 1 *N* sodium hydroxide solution. After occasional shaking in a water-bath at 80°C for 30 min, addition of 5 ml of 12% zinc sulphate solution and cooling to room temperature, the extracted solution was filtered and diluted accurately to 100 ml with distilled water. A 1-ml volume of the filtrate was placed in a test-tube and 1 ml of 5% silver sulphate solution, 3 ml of distilled water and (gently) 7 ml of sulphuric acid were added, followed by 0.1 ml of 2-*sec.*-butylphenol solution. The mixture was allowed to react as described above and analysed by GLC under the described conditions. The con-

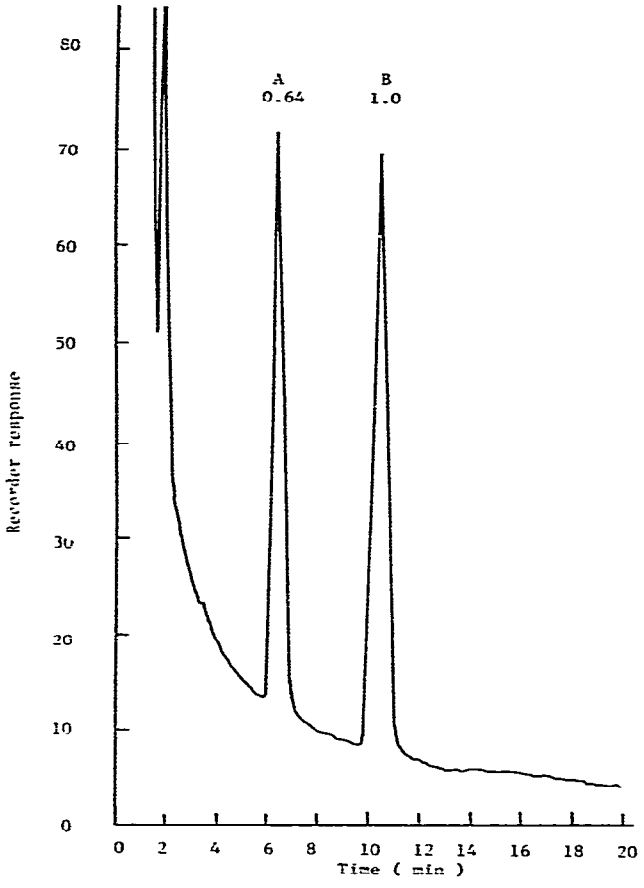

Fig. 1. Gas chromatogram of pentafluorobenzoylated extract of a standard reaction mixture (A) to which nitrate-nitrogen (0.05 μg) was added, with retention time relative to that of the internal standard (B). The pentafluoro derivative was obtained according to the described procedure. Sample size: 1 μl.

tents of nitrate in meat products and cheeses were determined from the peak height relative to that of the internal standard on the gas chromatograms and comparison with a calibration graph.

RESULTS AND DISCUSSION

Standard assay

As shown in Fig. 2, the calibration graph was rectilinear for 0.013–0.25 of NO_3-N per ml of reaction mixture. The average relative standard deviation of five determinations was 3.4% for 0.05 and 0.10 of NO_3-N and 4.5% for 0.25 μg, and the reproducibility was considered satisfactory.

Conditions for nitration of 2-sec.-butylphenol

The influence of sulphuric acid concentration on the nitration of 2-*sec.*-

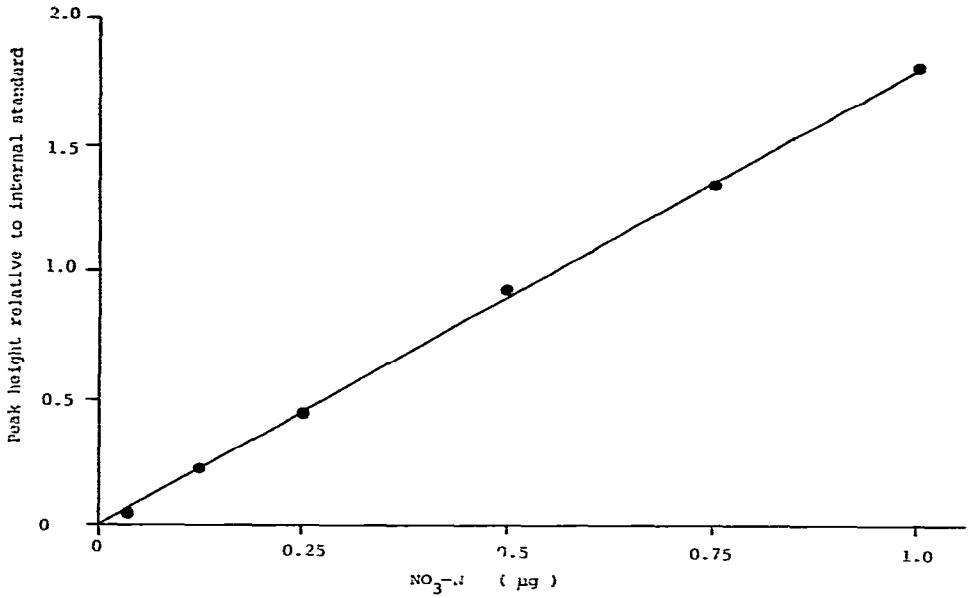

Fig. 2. Calibration graph for nitrate-nitrogen in the reaction mixture. The nitration and pentafluorobenzoylation and the subsequent GLC analysis were performed according to the described procedures. Sample size: 1 μl. Column temperature: 210°C. Nitrogen flow-rate: 75 ml/min.

butylphenol with nitrate to form the nitrated compound was studied by mixing 1.0 μg of NO_3-N, 0.1 ml of 5% 2-*sec.*-butylphenol solution, 50 mg of silver sulphate and 12 ml of sulphuric acid of various concentration for 15 min at room temperature. The results are shown in Fig. 3. In the sulphuric acid concentration range 55–75%, a constant peak height was obtained, but above a concentration of 80% the nitration yield of 2-*sec.*-butylphenol decreased. The use of a high concentration of sulphuric acid is dangerous and inconvenient for practical analyses. In our procedure, if 7 ml of sulphuric acid are added, the concentration of sulphuric acid is about 57.2%. If we assume that 1 mol of 2-*sec.*-butylphenol reacts with 1 mol of potassium nitrate, then 10.7 μg of 2-*sec.*-butylphenol are required for 7.2 μg of potassium nitrate (1.0 μg of NO_3-N). The relative yields of the nitro compound for various amounts of 2-*sec.*-butylphenol added to 7.2 μg of potassium nitrate in a total of 5 ml of solution were 95.8% for 0.5 mg of 2-*sec.*-butylphenol, 99.5% for 1 mg and 100% for 3, 5, 10, 15, 20 and 25 mg at room temperature with a reaction time of 15 min. To some extent, therefore, addition of 2-*sec.*-butylphenol in excess gave reasonable results, and in practice 0.1 ml of 5% reagent solution was added.

The optimal reaction time was investigated by mixing 1.0 μg of NO_3-N, 0.1 ml of 5% 2-*sec.*-butylphenol and 7 ml of sulphuric acid. After reaction, the mixture was analysed by GLC according to the described procedure. The production of the nitro derivative of 2-*sec.*-butylphenol with time is shown in Fig. 4. A reaction time of at least 5 min at room temperature is required, and in practice 15 min was used.

The production of the nitro compound at several temperatures was studied by mixing 1.0 μg of NO_3-N and 0.1 ml of 5% 2-*sec.*-butylphenol in a total volume of 5

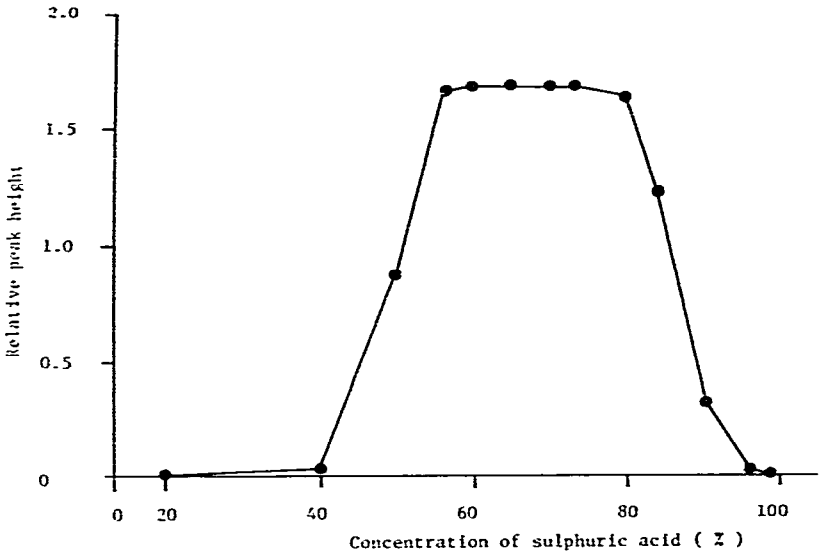

Fig. 3. Optimal concentration of sulphuric acid for the nitration of 2-*sec.*-butylphenol. To 1.0 μg of nitrate-nitrogen were added 0.1 ml of 5% 2-*sec.*-butylphenol solution, 50 mg of silver sulphate and 12 ml of various concentrations of sulphuric acid with reaction at room temperature for 15 min. The product was analysed by GLC according to the described procedure.

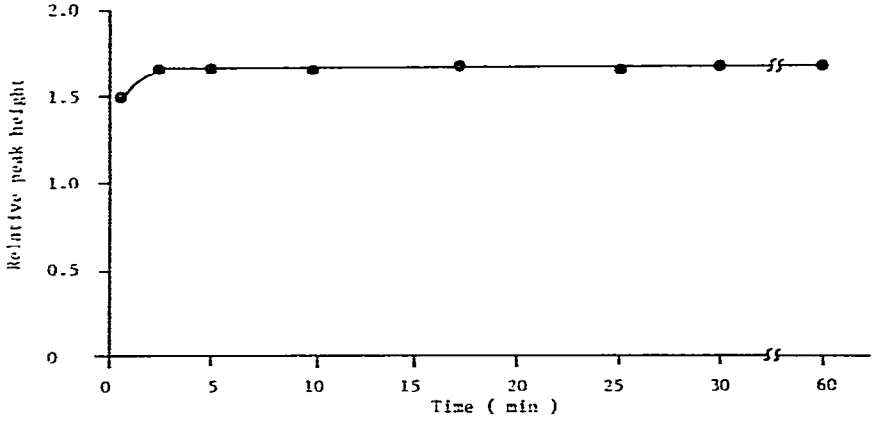

Fig. 4. Effect of reaction time on the nitration of 2-*sec.*-butylphenol. To 1.0 μg of nitrate-nitrogen were added 0.1 ml of 5% 2-*sec.*-butylphenol solution and 1 ml of 5% silver sulphate solution in a total volume of 5 ml, followed by 7 ml of sulphuric acid. The mixture was reacted with occasional shaking at room temperature. After nitration, the pentafluorobenzoylated extract was analysed by GLC according to the described procedure.

ml, followed by 7 ml of sulphuric acid. After reaction the mixture was analysed by GLC according to the described procedure. The relative yields obtained after reaction for 15 min were 100% at 0, 10, 40 and 60°C, 99.6% at 80°C and 94.0% at 100°C. Therefore, the reaction was performed at room temperature.

Pentafluorobenzoylation of the nitro compound of 2-sec.-butylphenol

Phenol benzoates are formed by the Schotten–Baumann reaction from the phenol and benzoyl chloride in the presence of an alkaline medium. The influence of sodium carbonate concentration on the reaction of the nitro derivative of 2-*sec.*-butylphenol with PFB-Cl to form the phenol benzoate was studied by mixing 20 μl of PFB-Cl, and the results are shown in Fig. 5. In the range 2.0–7.0%, a constant relative peak height is obtained, but above 7.5% the formation of phenol benzoate gradually decreased. On the other hand, other alkaline solutions such as sodium hydroxide and potassium hydroxide did not give symmetrical peaks on the chromatogram and sodium hydrogen carbonate did not form the phenol benzoate. Therefore, in practice 10 ml of 5% sodium carbonate solution is used. The optimal amount of PFB-Cl for the formation of phenol benzoate was investigated after proceeding to the nitration stage as described above using 1.0 μg of NO_3-N. The relative yields obtained after 5 min were 92.8% for 1 μl and 100% for 5, 10, 50 and 100 μl. Hence the use of 20 μl of PFB-Cl was adopted. On the other hand, the reaction proceeds fairly rapidly and when 20 μl of PFB-Cl were added to 10 ml of 5% sodium carbonate solution after nitration with the described procedure using 1.0 μg of NO_3-N, the yield of the phenol benzoate reached 100% within 5 min.

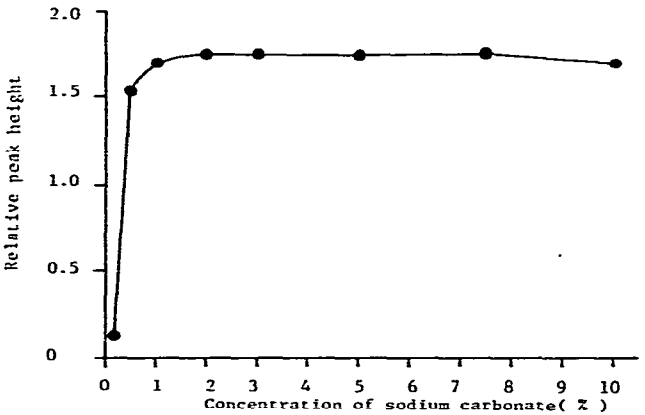

Fig. 5. Effect of sodium carbonate concentration on the pentafluorobenzoylation of nitrated 2-*sec.*-butylphenol. Reaction at room temperature for 5 min. Sample size for GLC: 1 μl.

Extraction

Various solvents were tried for the extraction of the nitrated derivative of 2-*sec.*-butylphenol and its PFB derivative, as shown in Table I. When benzene, toluene, xylene, chloroform, dichloromethane and carbon tetrachloride were used for the nitrated derivative of 2-*sec.*-butylphenol, the extraction yields were high, but with *n*-hexane it was low. Toluene was selected for the extraction of the nitrated derivative of 2-*sec.*-butylphenol because it did not cause co-extraction from the meat products and cheese and did not form an emulsion with water; *n*-hexane was used for the extraction of the PFB derivative for the same reason.

TABLE I

OPTIMAL SOLVENT FOR EXTRACTION OF NITRATED 2-*sec.*-BUTYLPHENOL (A) AND ITS PENTAFLUOROBENZOYL DERIVATIVE (B)

Reaction and GLC conditions as in the described procedure. The reaction mixture contained 5% 2-*sec.*-butylphenol (0.1 ml) and nitrate-nitrogen (1.0 μg).

Solvent	*Relative peak height (%)*	
	Compound A	*Compound B*
Benzene	100	100
Toluene	100	100
Xylene	99.5	100
Chloroform	99.3	100
Dichloromethane	93.1	100
Carbon tetrachloride	93.1	100
n-Hexane	48.0	100

Interferences

Potassium nitrate can be extracted from meat products and cheese with an alkaline solution. To investigate the effect of preservatives such as sorbic acid, benzoic acid, butylhydroxyanisole, butylhydroxytoluene, dehydroacetic acid and sodium nitrite on the determination, 1.0-μg portions of NO_3-N were added to 50–100 μg of various preservatives, and each mixture was analysed by GLC after the nitration and pentafluorobenzoylation stages as described procedure. As shown in Table II, none of them had much effect on the determination. On the other hand, the interference from nitrite can be prevented by using sulphamic acid if large amounts of nitrite are present, as reported by many investigators[9–11,17,18,20,22] (Table II). Therefore, a further clean-up stage is not needed. The influence of various amounts of chloride ions as another possible interferent was studied by mixing 1.0 μg of NO_3-N with the same reaction conditions as in the described procedure. As shown in Table III, chloride decreased the recovery of NO_3-N, as reported by many investigators[9–11,17,18,20–22]. However, this is also prevented by addition of silver sulphate solution (Table III). Therefore, in practice 1 ml of 5% silver sulphate solution is used. On the other hand, an addition of silver sulphate in the range 2.5–100 mg did not affect the recovery of NO_3-N. As shown in Fig. 6, the pentafluorobenzoylated extract obtained from various meat products and cheese gave gas chromatograms with good peak characteristics.

Gas chromatographic sensitivity

Columns containing 5% (w/w) of SE-30, DC-200, QF-1, OV-17, OV-101, OV-225 and XE-60 on Chromosorb W HP were tested. Except with OV-225, the columns showed the peak of the PFB derivative of the nitrated compound of 2-*sec.*-butylphenol; particularly good peak characteristics and sensitivity were achieved with OV-17 under the conditions described above. A high temperature and a short column were preferable for the GLC of the PFB derivative of the nitrated derivative of 2-*sec.*-butylphenol. At 200°C, a 1.5-m column containing OV-17 on Chromosorb W HP gave a good gas chromatogram, the retention time of the PFB derivative of the

TABLE II

INFLUENCE OF FOOD ADDITIVES ON RECOVERY OF NITRATE-NITROGEN

Each amount of food additive was added to a mixture of 1.0 μg of NO_3-N, 1 ml of 5% silver sulphate solution and 0.1 ml of 5% 2-*sec.*-butylphenol solution. Reaction and GLC conditions as in the described procedure.

Additive	*Amount added (μg)*	*Recovery of NO_3-N (%)*
Sorbic acid	50	100
	100	100
Benzoic acid	50	100
	100	99.8
Dehydroacetic acid	50	100
	100	99.7
Butyl *p*-hydroxybenzoate	50	100
	100	99.4
Butylhydroxyanisole	50	100
	100	98.9
Butylhydroxytoluene	50	100
	100	99.3
Sodium nitrite	50	98.8
	100	93.5

TABLE III

INFLUENCE OF CHLORIDE IONS ON RECOVERY OF NITRATE-NITROGEN

Each amount of chloride ion was added to a mixture of 1.0 μg nitrate-nitrogen and 0.1 ml of 5% 2-*sec.*-butylphenol solution. Reaction and GLC conditions as in the described procedure.

Chloride ion added (mg)	*Recovery (%)**
0	100
0.5	90.3 (100)
1.0	84.2 (100)
3.0	60.5 (100)
5.0	56.3 (100)
10.0	52.8 (100)
20.0	34.4 (63.7)
30.0	7.2 (16.7)

* Values in parentheses are recoveries after addition of 1 ml of 5% silver sulphate solution.

nitrated derivative of 2-*sec.*-butylphenol relative to that of the internal standard being 0.64. After pentafluorobenzoylation, the *n*-hexane phase extracted according to the described procedure should be injected into the gas chromatograph as soon as possible; with refrigeration, the sample was stable for at least 12 h, but after 24 h the content of the PFB derivative decreased to 90%.

Application and recoveries

Nitrate added to 10-g samples of pork sausage, fish sausage, meat ham, salami sausage, pollack roe, corned beef (canned) and cheese, chopped and then ground with

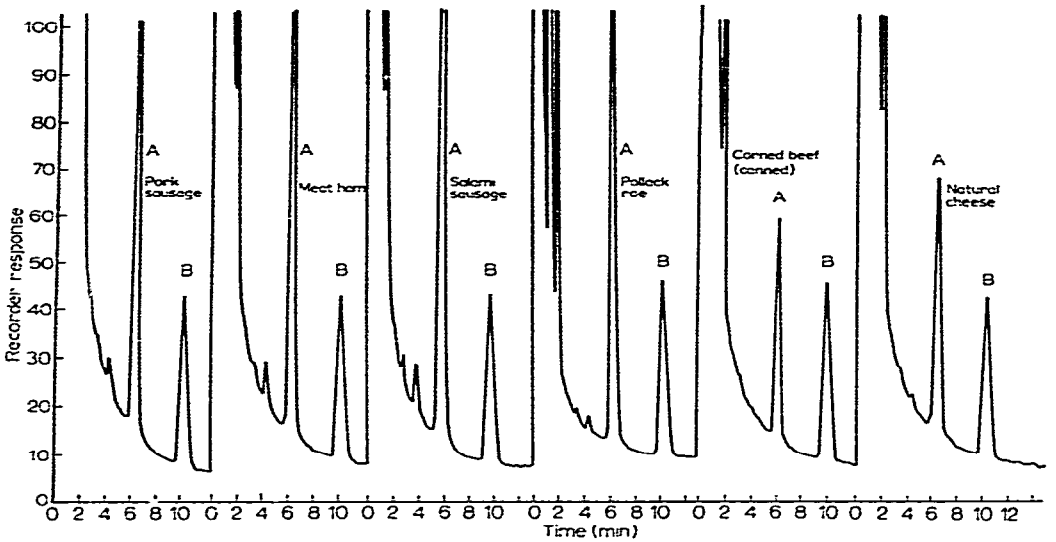

Fig. 6. Gas chromatograms of pentafluorobenzoylated extracts of various meat products and cheeses. Sample size: 1 μl. Peaks: A, pentafluorobenzoyl ester of 4-nitro-2-*sec.*-butylphenol; B, DDE.

a porcelain pestle and mortar, was determined by the proposed method. The recoveries of 5 and 10 ppm of nitrate-nitrogen, given in Table IV, ranged from 96.8 to 99.0% for 5 ppm and from 94.7 to 98.6% for 10 ppm. The detection limit was 0.07 ppm.

Identification of the nitrated compound of 2-sec.-butylphenol and its PFB derivative

To obtain the nitrated derivative of 2-*sec.*-butylphenol, 0.51 g of potassium nitrate and 0.75 g of 2-*sec.*-butylphenol (molar ratio 1:1) were added to 10 ml of *ca.* 57% sulphuric acid and the mixture was allowed to stand with occasional shaking for 15 min at room temperature; when the reaction was complete, the mixture was extracted with toluene. After washing with distilled water and drying with 0.1 g of

TABLE IV

PERCENTAGE RECOVERIES OF NITRATE ADDED TO VARIOUS MEAT PRODUCTS AND CHEESES AT THE 5 AND 10 ppm LEVELS

Each result is the average of four determinations.

Sample	*Amount of nitrate-nitrogen added (μg)*	
	50	*100*
Pork sausage	98.4	97.6
Fish sausage	96.8	98.3
Meat ham	98.4	98.2
Salami sausage	98.9	97.4
Pollack roe	97.8	96.4
Corned beef (canned)	97.1	95.9
Natural cheese	99.3	98.6
Processed cheese	96.8	94.7

anhydrous sodium sulphate, the toluene extract was evaporated to dryness. The residue was dissolved in 1 ml of acetone and portions of the acetone solution were spotted on a thin-layer plate (20 × 20 cm) pre-coated with a 0.3-mm layer of silica gel (Merck, Darmstadt, G.F.R.). Development was carried out with *n*-hexane in an equilibrated tank; the R_F values of the two yellowish bands were 0.21 and 0.98. The

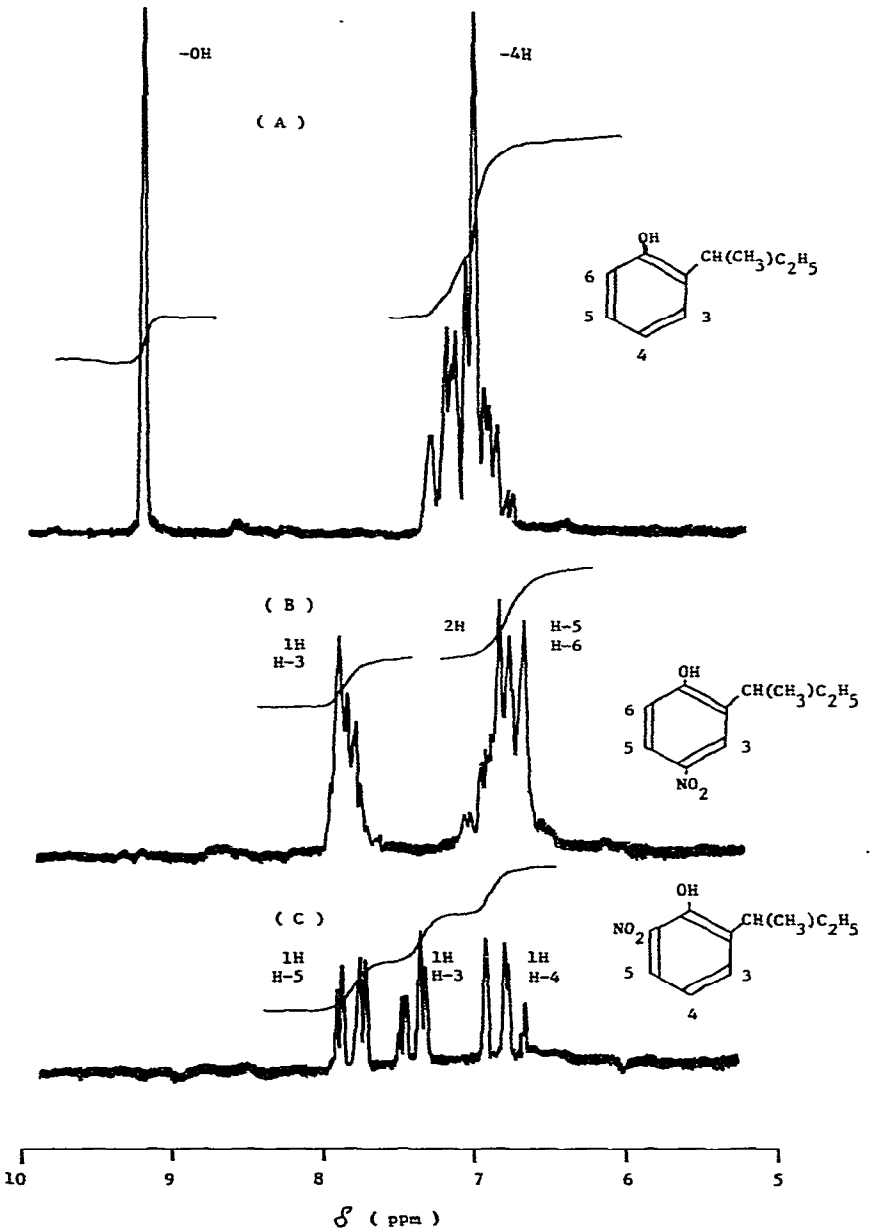

Fig. 7. NMR spectra of 2-*sec*.-butylphenol (A), 4-nitro-2-*sec*.-butylphenol (B) and 6-nitro-2-*sec*.-butylphenol (C) in dimethyl sulphoxide at 60 Hz.

spots were removed from the plate and dissolved in methanol, then filtered to separate the sample substances from silica gel. The filtrates were evaporated to dryness with a stream of dry nitrogen at room temperature and the residues were dissolved in 5 ml of dimethyl sulphoxide in order to identify the nitrated derivatives of 2-*sec.*-butylphenol by NMR spectroscopy. In the NMR spectrum of 2-*sec.*-butylphenol, signals appear at $\delta = 9.0$ (singlet peak; 1 H), which is indicative of a phenol group, and at $\delta = 6.5$–7.2 (multiplet; 4 H), which is indicative of an aromatic compound. As shown in Fig. 7, in the NMR spectrum of the nitrated compound ($R_F = 0.21$), signals appear at $\delta = 6.6$–6.9 (multiplet; 2 H), which is indicative of the C-5 and C-6 protons, and at $\delta = 7.7$–8.0 (singlet; 1 H), which is indicative of the C-3 proton; this suggests the replacement of the C-4 proton by a nitro group. On the other hand, in the NMR spectrum of the nitrated compound ($R_F = 0.98$), signals appear at $\delta = 6.65$–6.98 (triplet; 1 H), $\delta = 7.32$–7.58 (doublet; 1 H) and $\delta = 7.72$–7.94 (doublet; 1 H), which are indicative of C-4, C-3 and C-5 protons, respectively. This suggests the replacement of the C-6 proton by a nitro group (Fig. 7). Therefore, from these NMR spectra, we concluded that the product with $R_F = 0.21$ was 4-nitro-2-*sec.*-butylphenol and that with $R_F = 0.98$ was 6-nitro-2-*sec.*-butylphenol.

The two products were subjected to gas chromatography after pentafluorobenzoylation according to the described procedure. These PFB derivatives gave single peaks on the gas chromatogram with retention times of 0.64 ($R_F = 0.21$) and 0.61 ($R_F = 0.98$) relative to the internal standard. Although the peak obtained from the TLC band with $R_F = 0.21$ was in agreement with Fig. 1, the other product ($R_F = 0.98$) was not. In order to determine whether the PFB derivative of 4-nitro-2-*sec.*-butylphenol decomposed during the GLC process, the identity of the product was confirmed by GLC–MS. The mass spectrum of 2-*sec.*-butylphenol exhibited ion peaks at *m/e* 150 (M^+), 149 ($M^+ - H$), 132 ($M^+ - H_2O$), 122 ($M^+ - CO$) and 121 ($M^+ - CHO$). The fragmentation pattern of the PFB derivative of 4-nitro-2-*sec.*-butylphenol is shown in Fig. 8, with peaks at *m/e* 389 (M^+), 194 (C_6F_5CO) (a), 166 (a − CO) (b), 150 (b − NO) and 120 (b − NO_2). The parent peak (*m/e* 150) for 2-*sec.*-butylphenol and that at *m/e* 389 for the PFB derivative of 4-nitro-2-*sec.*-butylphenol correspond to the molecular weight of each compound. The shift of the peaks from *m/e* 150 to 121 for 2-*sec.*-butylphenol could be attributed to a characteristic phenol degradation

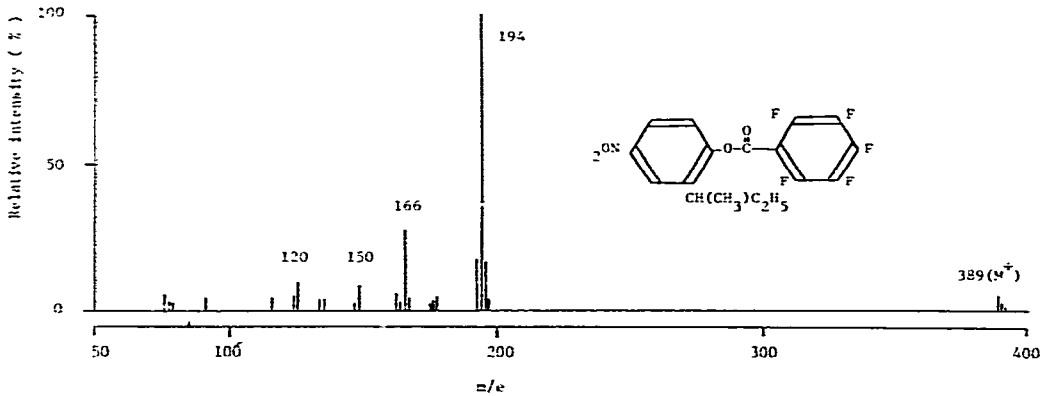

Fig. 8. Mass spectrum of pentafluorobenzoyl ester of 4-nitro-2-*sec.*-butylphenol.

compound. On the other hand, the shift of the peaks from *m/e* 389 to 194 for the PFB derivative could be attributed to initial degradation of the PFB group, and the subsequent shift from *m/e* 166 to 120 could be ascribed to the loss of a nitro group.

From this series of experiments, it was concluded that the PFB derivative of nitrated 2-*sec*.-butylphenol was the pentafluorobenzoyl ester of 4-nitro-2-*sec*.-butylphenol.

ACKNOWLEDGEMENT

We thank Mrs. Eri Inamura for her help in carrying out the NMR studies.

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